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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of Brian W. Ward
Serial No. : 09/610,935
Filed : July 6, 2000
Confirmation No. 5148

Art Unit 1634

For TARGET REAGENTS THAT ENHANCE REACTION-PRODUCT ANALYSIS

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

**DECLARATION OF PRIOR INVENTION IN THE UNITED STATES OR IN A NAFTA
OR WTO MEMBER COUNTRY TO OVERCOME CITED PATENT OR PUBLICATION
(37 C.F.R. § 1.131)**

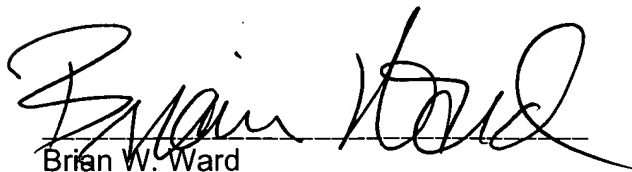
I, Brian W. Ward declare as follows:

1. I am an inventor of the subject matter claimed in the above-entitled United States patent application.
2. I am submitting this Declaration to establish completion of our invention in the U.S. before December 7, 1998, the filing date of U.S. Patent No. 6,153, 412.
3. The invention claimed in this application was reduced to practice in the United States before December 7, 1998.
4. Facts in support of this Declaration are attached hereto as Exhibit A. Exhibit A is a true and correct copy of experimental note book pages, data sheets, and graphs, each Bates-stamped as exhibit pages 00001-00038. Dates have been redacted from these pages but all such dates are before December 7, 1998.
5. The matter contained in Exhibit A is that upon which Figures 11, 12A, 12B, 13, 15, 16, and Example 1 of this application are based. Specifically, page 00003

corresponds to Figure 11; page 00008 corresponds to Figure 12A; page 00011 corresponds to Figure 12B; page 00012 corresponds to Figure 16; page 00029 corresponds to Figure 13; and page 00035 corresponds to Figure 15 of this application.

6. The matter contained in Exhibit A reflects embodiments of the claims of the present application. An aqueous reagent containing Taq DNA polymerase, anionic tracer dye, and a solute to increase the physical density, but not containing primer or nucleic acid polymer template, is referenced on page 0021 and 0038. Preparation of such reagent with acid red 1 (AR1) and acid violet 5 (AV5) anionic tracer dye is referenced throughout the provided pages. An 85/15 proportion of AV1/AV5 is referenced on pages 00014, 00016, 00017, 00020, 00021, A solute (specifically, glycerol) that increases the physical density of the reagent is referenced on pages 00001, 00012, 00013, and 00038. A reagent with Taq DNA polymerase, anionic tracer dye, solute, no primer or template, and a density of at least 1.01 g/cm^3 (and specifically, 1.14 g/cm^3) is referenced on page 0038. Optical densities between 5 and 500 in the reagent are referenced on pages 00016, 00021, and 00038. Taq DNA polymerase concentrations between 0.033 and 10 units/ μl of reagent are referenced on pages 00015, 00021, 00026, 00034, and 00038. As such, Exhibit A evidences our reduction to practice of the invention claimed in the application prior to December 7, 1998.

7. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.


Brian W. Ward


Date

50% glyc	50% gly	AV5	H ₂ O
% gly		9 mg/ml	
5	10	25	65
4.5	9	↓	66
4	8		67
3.5	7		68
3	6		69
2.5	5		70

load OK to 1.4% or lower

1ml 2.5% glycerol 1X PCR

50 μ l 50% glyc, 100 μ l 10X PCR, 850 H₂O

in PCR ~ 0.8 mg/ml \rightarrow Enz 16 mg/ml

PCR Yield

36 reactions - ²⁰~~40~~ μ l ea -

20 μ l dge, 20 μ l ~~EX~~ PCR mix

PCR MIX - (²⁰⁰~~800~~ μ l) 10X - ⁸⁰~~400~~ μ l

10mM dNTP's - ⁸~~16~~ μ l, α ^{2.5}~~5~~ μ l 32 P dCTP

PMRS ⁴⁰~~80~~ μ l ea, Temp ⁴⁰~~80~~ μ l, Tacy 40
 H_2O 149.5

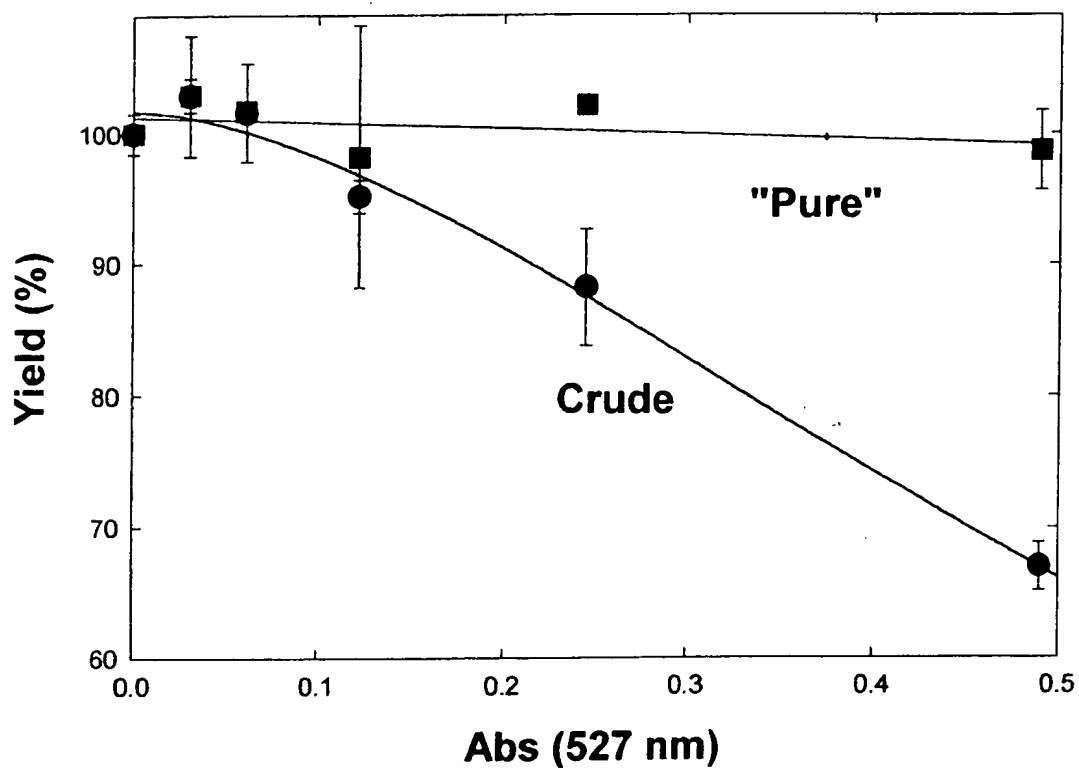
1	7	12
A AV5	0 AV	0
B AR1	0 AR5	0
C AV/AR	0 AV/AR	0

AV5, AR1 + ¹⁰~~32~~ μ l H_2O $\rightarrow A_{max} = 100$

AV/AR 8 ea + 4 H_2O $\rightarrow Total = 100$

Finan David

**PCR yield of crude vs. pure (desalted)
Acid Violet 5 containing reactions.
Template/primer= lambda 500mer,
20 cycles at 94/55/72°C, 1 min each.
Abs=0.5 approx. 0.5 mg/ml of crude dye.**



Purified "AV5"

USER: 1 ID:GONEHEAD PRESET TIME: 1.00
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N R6232:N
 H#: 0 ADD:N BCF:N RCM:N
 CHANNEL 1-LL: 0 UL:1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
 DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:0 1.00000
 HALF LIFE(DAYS):N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	ERR
1	**	1	271360.00	1.72	0.05	0.22	
2	**	2	363120.00	1.48	0.05	0.60	
3	**	3	374700.00	1.46	0.05	0.97	
4	**	4	414120.00	1.39	0.05	1.35	
5	**	5	421720.00	1.38	0.05	1.72	
6	**	6	404520.00	1.41	0.05	2.10	
7	**	7	260940.00	1.75	0.05	2.47	
8	**	8	338120.00	1.54	0.05	2.84	
9	**	9	381780.00	1.45	0.05	3.21	
10	**	10	393240.00	1.43	0.05	3.58	
11	**	11	395820.00	1.42	0.05	3.96	
12	**	12	393980.00	1.42	0.05	4.33	
13	**	1	400560.00	1.41	0.05	4.80	
14	**	2	404860.00	1.41	0.05	5.17	
15	**	3	418380.00	1.38	0.05	5.55	
16	**	4	404260.00	1.41	0.05	5.92	
17	**	5	405360.00	1.40	0.05	6.30	
18	**	6	393660.00	1.43	0.05	6.67	
19	**	7	383680.00	1.44	0.05	7.05	
20	**	8	406880.00	1.40	0.05	7.42	
21	**	9	362080.00	1.49	0.05	7.80	
22	**	10	403980.00	1.41	0.05	8.17	
23	**	11	412540.00	1.39	0.05	8.55	
24	**	12	148290.00	1.64	0.10	8.97	

0.98/2 Abs

Bulk

0.98/2 Abs

Pure

From Page No. _____

Mg AR1 / AVE

1A → 12

Mg Dyes.

2A → 12

10⁴ Dyes + MgCl₂Mg Dyes - 10⁴ Dye @ A_{max} = 100, 10⁴ rxnMgCl₂ + rxn.[MgCl₂]1 → 6 [MgCl₂] + 1X PCR4X MgCl₂ 40, 32, 24,
16, 8 mM

100 ⁴ MgCl ₂	H ₂ O	96	96.8	97.6	98.4	99.2
1M MgCl ₂	4	3.2	2.4	1.6	0.8	

To Page 1

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Date

TTT

Project No. _____

TITLE _____

Book No. _____

From Page No. _____

Zn, Ca & Mg AR π

2X mix 225 μ l,

10X 45 μ l, dNTP's 4.5 μ l

Primer/Temp 22.5 $^{\circ}$ C

α^{32} P dCTP 4 μ l Tag 22.5

H₂O 83.6

~~PyPI EDTA Time course~~

~~comp² (cutting)~~

Bulk \rightarrow

	10X	13.2	13.2
	PA20	1	1
	Origo	25.1	25.1 13.2
	H ₂ O	80.7	92.6

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TITLE _____ Book No. _____

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Zn, Ca { Mg AR π

2X mix 225 μ l,

10X 45 μ l, dNTP's 4.5 μ l

Prim/Temp 22.5 $^{\circ}$ C

α^{32} P dCTP 4 μ l Tag 22.5

H₂O 83.6

~~Fig 1 EDTA Time course~~

~~comp 2 (cutting)~~

~~Back~~

~~→ PA20~~

~~10X~~

~~T₂₀~~

~~13.2~~

~~13.2~~

~~1~~

~~1~~

~~Oligo~~

~~25.1~~

~~25.1 13.2~~

~~H₂O~~

~~80.7~~

~~92.6~~

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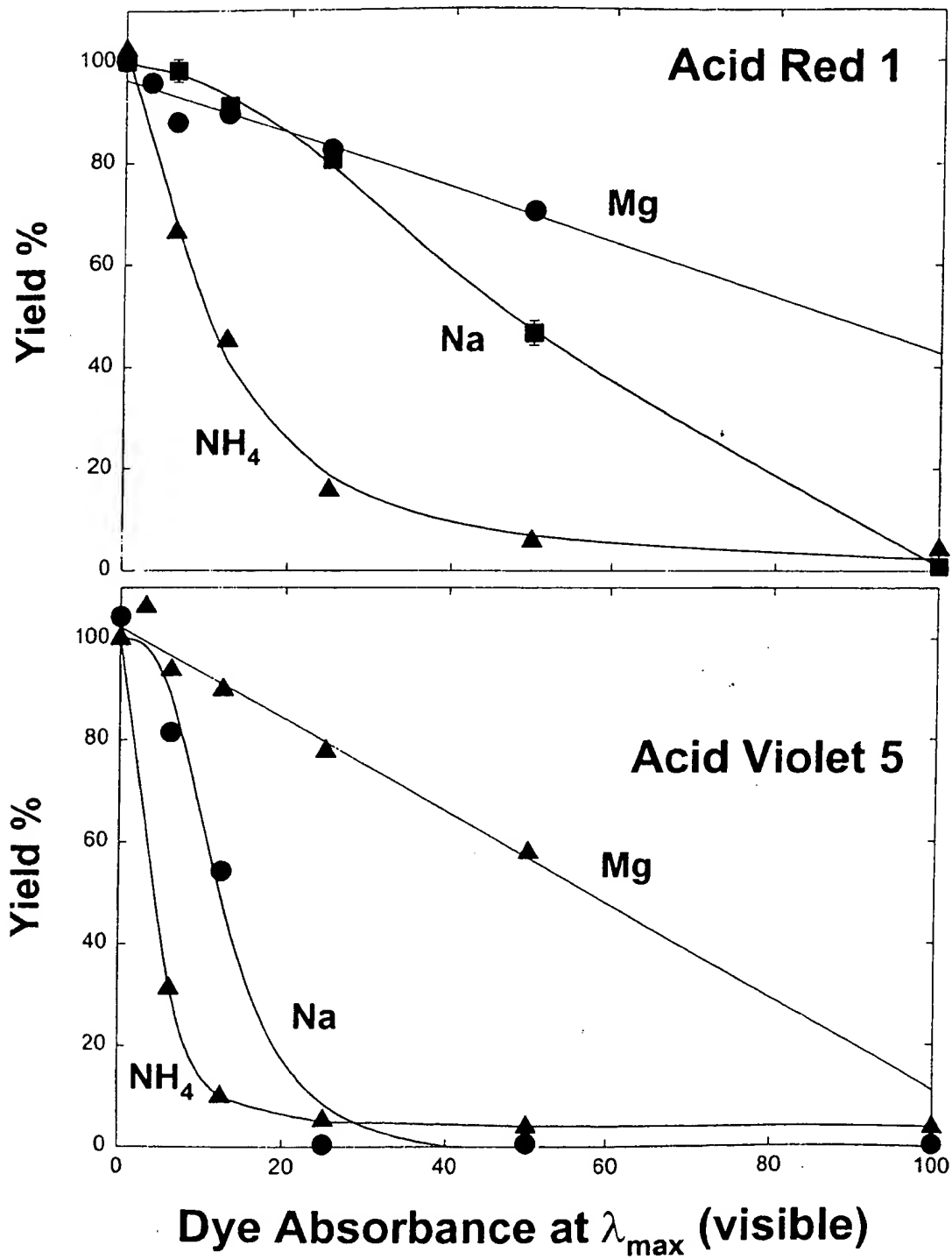
Date

Recorded by

00007

Simon H. H. Q.

Counterion dependence of PCR yield



USER: 1 ID: BONEHEAD PRESET TIME: 1.00
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N RS232: N
 H#: 0 ADD: N GCF: N RCM: N
 CHANNEL 1-LL: 0 UL: 1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
 DATA CALD: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000
 HALF LIFE(DAYS): N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	ERR
1	**	1	1234.00	5.58	1.00	1.18	70
2	**	2	904.00	6.65	1.00	2.52	8
3	**	3	967.00	6.43	1.00	3.87	6 + MgCl ₂
4	**	4	1076.00	6.10	1.00	5.21	4 mM
5	**	5	1863.00	4.63	1.00	6.55	2
6	**	6	16424.62	1.94	0.65	7.53	0
7	**	7	474320.00	1.30	0.05	7.90	50
8	**	8	555580.00	1.20	0.05	8.28	25
9	**	9	604140.00	1.15	0.05	8.65	12.5
10	**	10	593400.00	1.16	0.05	9.03	4.25
11	**	11	646100.00	1.11	0.05	9.40	3.125
12	**	12	674380.00	1.09	0.05	9.78	0
13	**	1	1531.00	5.11	1.00	11.12	50
14	**	2	1454.00	5.25	1.00	12.52	25
15	**	3	1937.00	4.54	1.00	13.81	12.5
16	**	4	939.00	6.53	1.00	15.20	4.25
17	**	5	1297.00	5.55	1.00	16.54	3.125
18	**	6	1071.00	6.11	1.00	17.88	0
19	**	7	386520.00	1.44	0.05	18.26	50
20	**	8	520720.00	1.24	0.05	18.63	25
21	**	9	601440.00	1.15	0.05	19.01	12.5
22	**	10	627540.00	1.13	0.05	19.38	4.25
23	**	11	711500.00	1.06	0.05	19.77	3.125
24	**	12	669760.00	1.09	0.05	20.13	0

ARI-NH₄
 Mg ARI A₅₃₂
 +MgCl₂
 AV5-NH₄
 A₅₂₇
 AV5-Mg

USER: 1 ID: BONEHEAD PRESET TIME: 1.00
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N RS232: N
 ##: 0 ACC: N OCF: N RCM: N
 CHANNEL 1-LL: 0 UL: 1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
 DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000
 HALF LIFE(DAYS): N

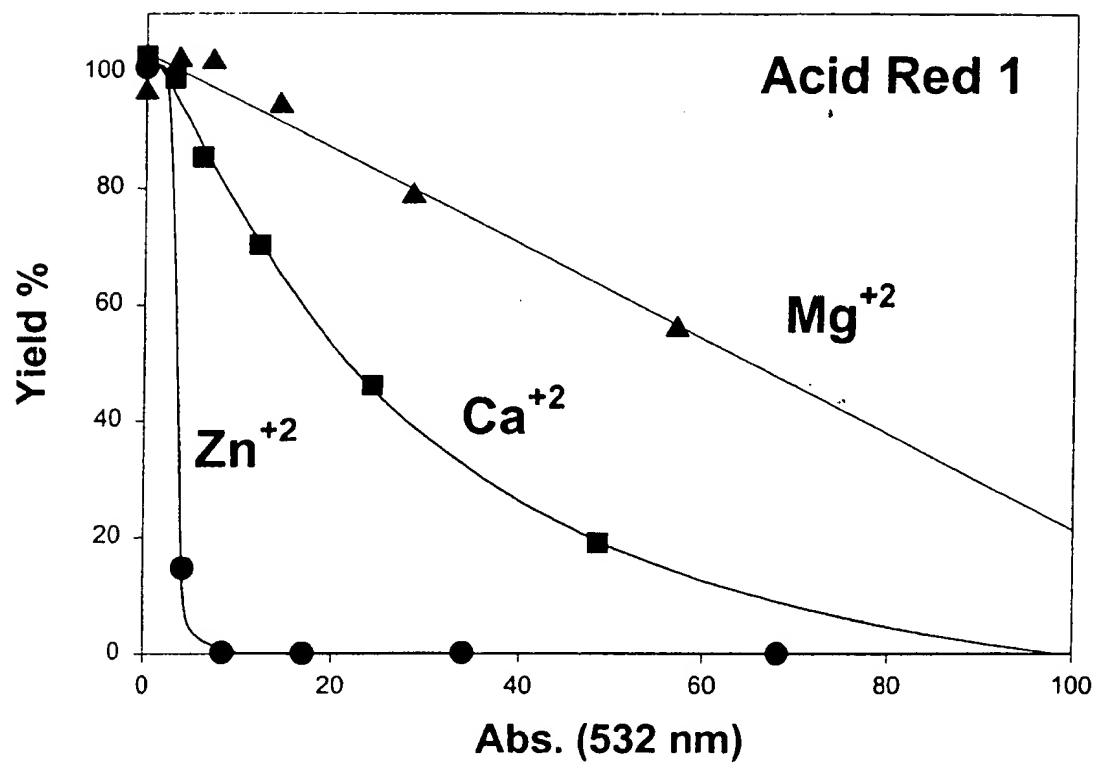
SAM	PDS	CH	CPM	2SIG%	TIME	EL TIME	EPR
1	**	1	662.00	7.77	1.00	1.18	
2	**	2	1306.00	5.53	1.00	2.50	
3	**	3	3133.00	3.57	1.00	3.84	
4	**	4	8838.00	2.13	1.00	5.18	
5	**	5	12747.50	1.98	0.80	6.33	
6	**	6	19934.54	1.91	0.55	7.21	
7	**	7	814.00	7.01	1.00	8.55	
8	**	8	780.00	7.16	1.00	9.88	
9	**	9	2486.00	4.01	1.00	11.23	
10	**	10	7301.00	2.34	1.00	12.56	
11	**	11	10994.74	1.96	0.95	13.61	
12	**	12	16646.15	1.92	0.65	14.83	
13	**	1	746.67	18.90	0.15	15.39	
14	**	2	1123.33	10.89	0.30	16.06	
15	**	3	888.89	10.00	0.45	16.80	
16	**	4	1795.00	6.09	0.60	17.83	
17	**	5	5274.74	2.83	0.95	19.12	
18	**	6	17731.67	1.74	0.80	20.07	
19	**	7	655.00	7.81	1.00	21.43	
20	**	8	670.00	7.73	1.00	22.77	
21	**	9	895.00	6.69	1.00	24.11	
22	**	10	1719.00	4.82	1.00	25.42	
23	**	11	5380.00	2.73	1.00	26.77	
24	**	12	17263.33	1.97	0.60	27.70	

ARI - NH₄

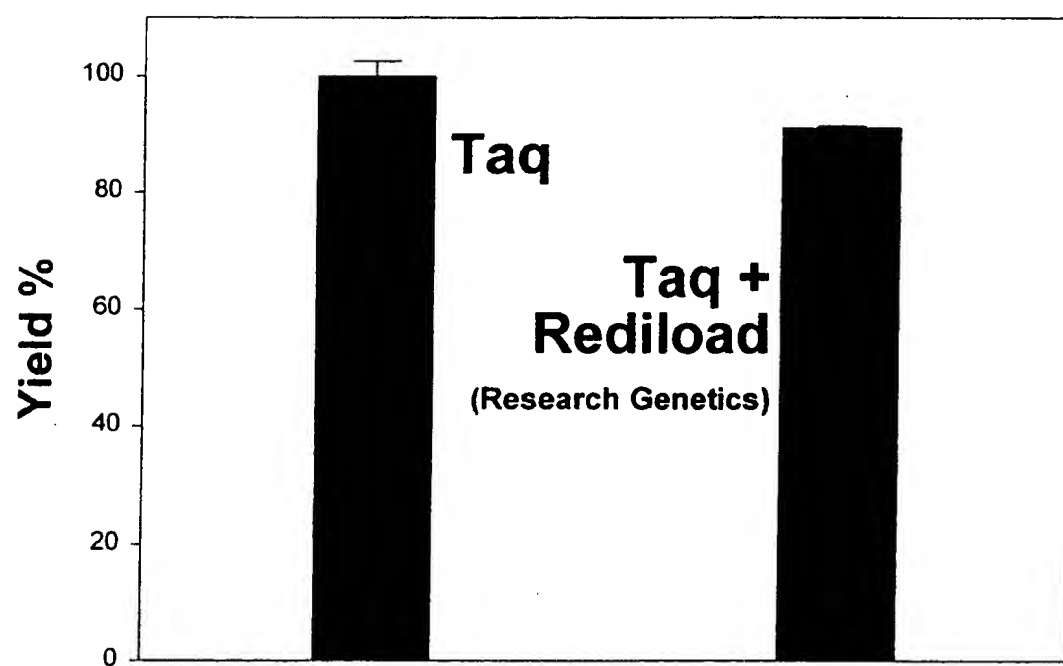
AV5 NH₄

Eric H. H.

Effect of divalent counter ion on PCR yield.



**Yield of λ 500mer for Taq
and Taq plus *Rediload***



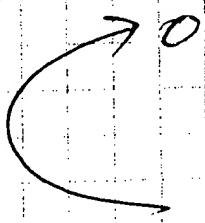
SER: 1 ID: BONEHEAD PRESET TIME: 1.00
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N RS232: N
 H#: 0 ADD: N QCF: N RCM: N
 CHANNEL 1-LL: 0 UL: 1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
 DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000
 HALF LIFE (DAYS): N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	ERR
1	**	1	153860.00	1.61	0.10	0.27	
2	**	2	370180.00	1.47	0.05	0.65	
3	**	3	563220.00	1.19	0.05	1.02	
4	**	4	684200.00	1.08	0.05	1.39	Ca
5	**	5	792840.00	1.00	0.05	1.77	
6	**	6	822840.00	0.99	0.05	2.15	
7	**	7	1180.00	5.82	1.00	3.49	
8	**	8	1840.00	4.66	1.00	4.81	
9	**	9	1136.00	5.93	1.00	6.13	Zn
10	**	10	1825.00	4.68	1.00	7.48	
11	**	11	118080.00	1.84	0.10	7.90	
12	**	12	607280.00	1.00	0.05	8.28	
13	**	1	448040.00	1.34	0.05	8.72	136.5
14	**	2	630220.00	1.13	0.05	9.09	67.9
15	**	3	754640.00	1.03	0.05	9.48	35.8
16	**	4	814480.00	0.99	0.05	9.85	16.43
17	**	5	817500.00	0.99	0.05	10.23	3.96
18	**	6	773140.00	1.02	0.05	10.62	0
19	**	7	727080.00	1.05	0.05	10.99	
20	**	8	728560.00	1.05	0.05	11.37	
21	**	9	732000.00	1.05	0.05	11.74	Redi Load.

From Page No. _____

5% AR1 / 15% AV5 titration

A _{TOT}	2X	V 5/4 → A _{TOT} = 500 (200 μl)	V Tag Strange Buffer
20	40	16	0
18	17.5	35	2
16	15	30	4
14	12.5	25	6
12	10	20	8
10	0	0	0



H₂O - compare yield to 5 μl

NXNS - 24 NXNS. 12 500mer,
12 5000mer

~~2X as pg 84~~

2X (150 μl) 10X 30 μl, 10 μl dNTP's 3,
Pms 6 μl ea Temp 15 μ,
α³²P dCTP 2 μl, Tag 3,
85 H₂O

To Page _____

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[Signature]

Date _____

Invented by

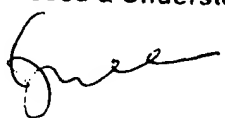
Recorded by

Date _____

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2X (137.5 μ l) 10X 27.5 μ l, pmrs 5.5 μ l
2 14 μ l, dNTP's 2.75 μ l,
³²P dCTP 1.5 μ l, ENZ 2.75 μ l
5 μ l/ μ l
H₂O 78

Witnessed & Understood by me,



Date

Invented by

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Date

To Page No. _____

From Page No. _____

Acid Red, AR1/AV5 mixes

24 rxns. 12 rxn ea pmr 20,
1

~~AR1/AV5~~ % AR1 100
82.5
65
0

Pmr / Temp $\lambda = \text{temp.}$

Pmrs @ 50X

2X - (275 μ l ea) 27.5
55 μ l 10X
pmrs 5.5 μ l ea, λ 27.5 μ l 14
dNTP's 2.75, 32P dCTP 1.5
ENZ ~~2.75~~ 5.5 μ l Tq @ 5.1
H₂O 156.5 2.75

2X dyes. 4 μ l + 46 μ l H₂O

0.8 μ l ~~500~~ A=500 pm rxn -

$\frac{10}{\text{rxn}} \leftarrow \frac{48 \mu\text{l}}{50}$

To Page

Witnessed & Understood by me,

[Signature]

Date

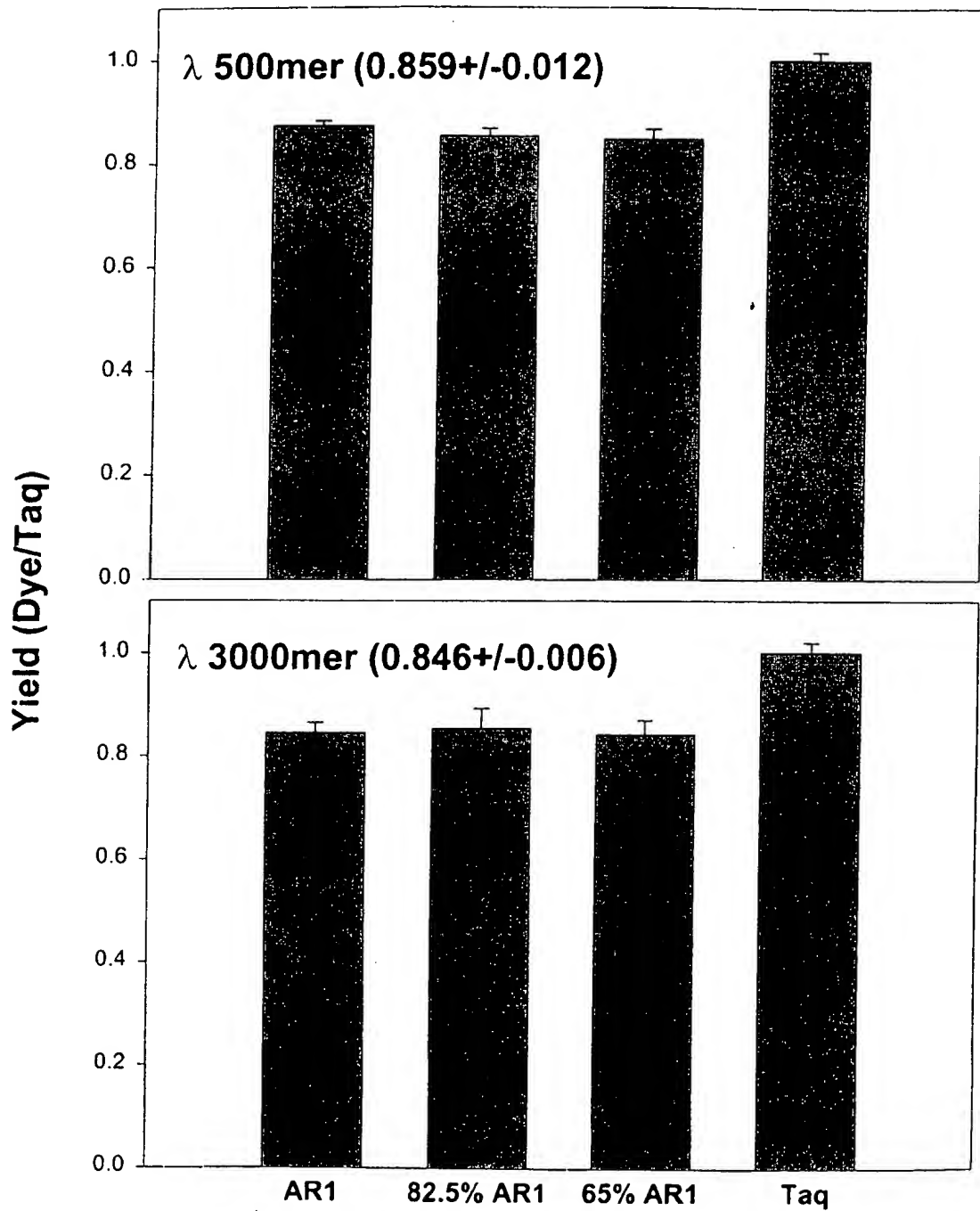
Invented by

Recorded by

Date

Shan Patel

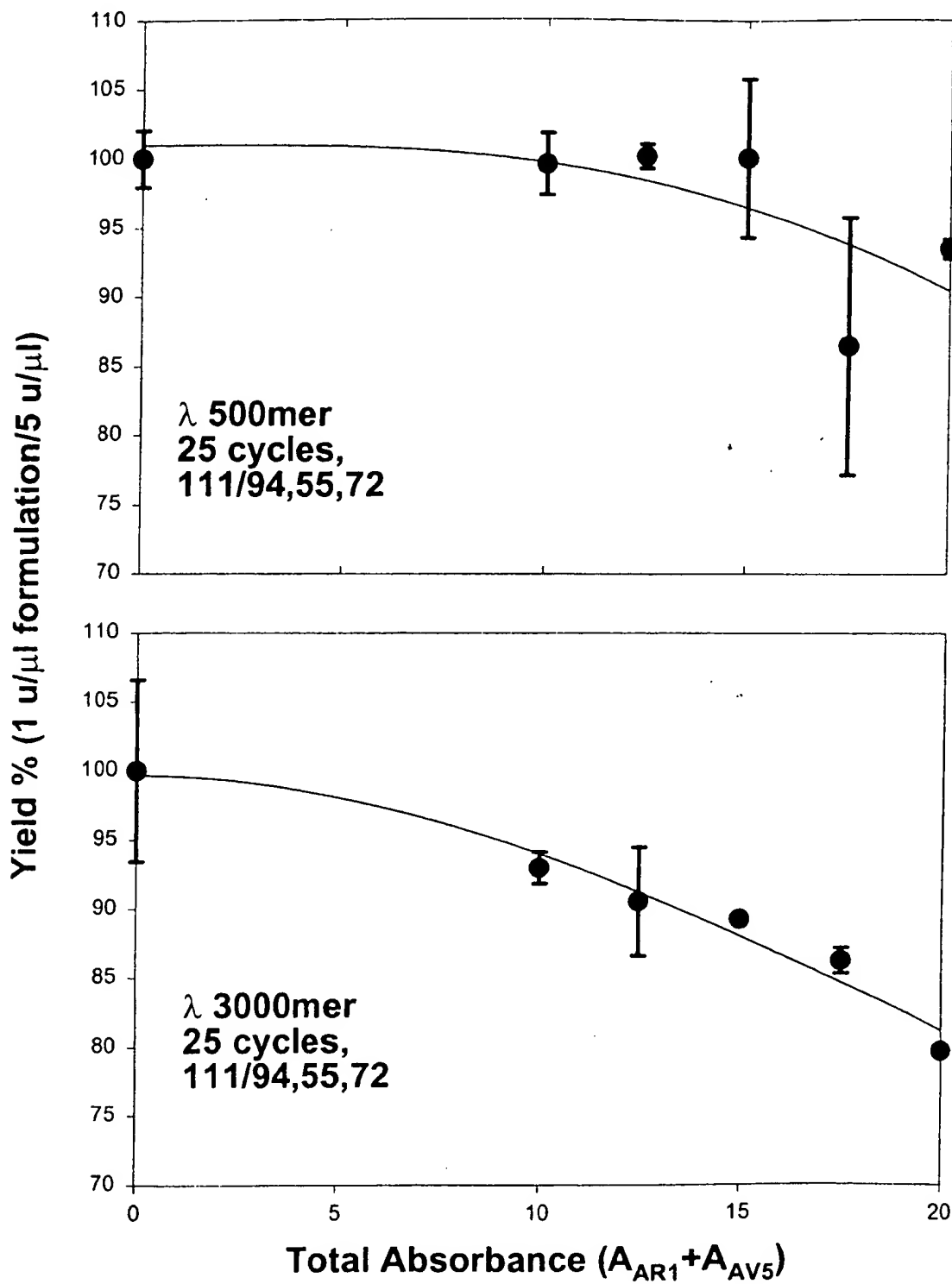
Acid Red 1, Acid Violet 5 mixes. $A_{527} + A_{532} = 20$.



USER: 1 ID: BONEHEAD PRESET TIME: 1.00
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N RS232: N
 H#: 0 AOC: N OCF: N RCM: N
 CHANNEL 1-LL: 0 UL: 1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
 DATA CALD: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000
 HALF LIFE(DAYS): N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVI	ERR
1	**	1	262680.00	1.75	0.05	0.22	100	
2	**	2	250680.00	1.79	0.05	0.59	82.5	
3	**	3	253920.00	1.77	0.05	0.96	65	
4	**	4	304120.00	1.62	0.05	1.33	0	
5	**	5	262360.00	1.75	0.05	1.69	100	
6	**	6	258880.00	1.76	0.05	2.06	82.5	
7	**	7	248000.00	1.80	0.05	2.42	65	
8	**	8	295900.00	1.64	0.05	2.80	0	
9	**	9	257720.00	1.76	0.05	3.16		
10	**	10	256520.00	1.77	0.05	3.52		
11	**	11	259260.00	1.76	0.05	3.89		
12	**	12	296440.00	1.64	0.05	4.27		
13	**	1	187260.00	1.46	0.10	4.73		
14	**	2	188460.00	1.46	0.10	5.16		
15	**	3	188200.00	1.46	0.10	5.58		
16	**	4	224580.00	1.89	0.05	5.95		
17	**	5	180940.00	1.49	0.10	6.38		
18	**	6	178800.00	1.50	0.10	6.80		
19	**	7	177840.00	1.50	0.10	7.23		
20	**	8	219380.00	1.91	0.05	7.59		
21	**	9	189330.00	1.45	0.10	8.02		
22	**	10	195870.00	1.43	0.10	8.44		
23	**	11	188910.00	1.46	0.10	8.87		
24	**	12	216100.00	1.92	0.05	9.23		
25	**	1	452580.00	1.33	0.05	9.67		
26	**	2	453440.00	1.33	0.05	10.04		
27	**	3	448500.00	1.34	0.05	10.42		
28	**	4	422080.00	1.38	0.05	10.79		
29	**	5	264560.00	1.74	0.05	11.16		
30	**	6	264000.00	1.74	0.05	11.52		
31	**	7	276780.00	1.70	0.05	11.88		
32	**	8	260840.00	1.75	0.05	12.24		
33	**	9	286240.00	1.67	0.05	12.62		
34	**	10	281540.00	1.69	0.05	12.98		
35	**	11	295420.00	1.65	0.05	13.34		
36	**	12	271680.00	1.72	0.05	13.70		

Shan V. Q.
PCR yields as a function 85% AR1 concentration.



85% AR1, 15% AV5

SER: 1 ID: BONEHEAD PRESET TIME: 1.00
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N RS232: N
 H#: 0 AQC: N QCF: N RCM: N
 CHANNEL 1-LL: 0 UL: 1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
 DATA CALC: CFM. UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000
 HALF LIFE(DAYS): N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	A	ERR
1	**	1	592900.00	1.16	0.05	0.22	20.	
2	**	2	509080.00	1.25	0.05	0.61	17.5	
3	**	3	663160.00	1.10	0.05	0.98	15.	
4	**	4	641960.00	1.12	0.05	1.36	12.5	
5	**	5	644420.00	1.11	0.05	1.73	10.	
6	**	6	627980.00	1.13	0.05	2.11	0	
7	**	7	599180.00	1.16	0.05	2.48	20	
8	**	8	592960.00	1.16	0.05	2.86	17.5	
9	**	9	611580.00	1.14	0.05	3.23	15	
10	**	10	634020.00	1.12	0.05	3.61	12.5	
11	**	11	624500.00	1.13	0.05	3.98	10	
12	**	12	646420.00	1.11	0.05	4.36	20	
13	**	1	505300.00	1.26	0.05	4.82	17.5	
14	**	2	544660.00	1.21	0.05	5.20	15	
15	**	3	568020.00	1.19	0.05	5.57	12.5	
16	**	4	593700.00	1.16	0.05	5.95	10	
17	**	5	586300.00	1.17	0.05	6.32	0	
18	**	6	606820.00	1.15	0.05	6.70	20	
19	**	7	508140.00	1.25	0.05	7.07	17.5	
20	**	8	553040.00	1.20	0.05	7.45	15	
21	**	9	19154.54	1.95	0.55	8.33	12.5	
22	**	10	558160.00	1.20	0.05	8.71	10	
23	**	11	596720.00	1.16	0.05	9.08	0	
24	**	12	665980.00	1.10	0.05	9.46	0	

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0.85 AR1, ^{0.15}AV5 @ A=500, 37.5

1 u/ml Tags.

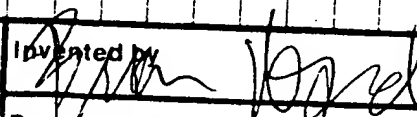
Abs	500	375
AR1 (615.6)	207	155
AV5 (541)	41.6	31.2
STORAGE Buf	51.4	113.8

Witnessed & Understood by me,



Date

Invented by



Recorded by

Date

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1500 & 3000 mer (Mg) app

Dye/CTL

3/4 Dye

STAGE 20 μ l
+ 180 H_2O ~~20~~2X E mixes. 275 μ l ER.PMRS 11 μ l ER, λ 13.5 μ l10 mM dATP'S 5.5 μ ld³²P dATP 3 μ l, 5.5 μ l225.5 H_2O (54) λ 1 (anchor)
 λ (1500)
 λ (3000)

1	2	3	4	5	6	7	8	9	10	11
15	12.5	10	8	7	6	5	4	3	2	1

10X [Mg]

10X + Mg

10X - Mg

20

37.33

2.67

2.87

5.33

4.67

13.33

14.67

17.33

37.33

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White ~~2 μ l~~ 10 μ l 2X
 ~~8 μ l~~ 10X 10 μ l 2X
 H₂O Buffer } x 4
 10 μ l 2X Enz mix }

Red 15 ~~2 μ l~~ 1 mM Mg 10X
 ~~8 μ l~~ dye } x 4
 10 μ l 2X Enz mix }

Red 25 2 μ l 8 mM Mg 10X
 8 μ l Dye } x 4
 10 μ l 2X Enz mix }

2X 500 mer (135 μ l) 2.7 μ l Tag. 5.4 μ l

13.5 λ 2.7 dATP's

2 μ l ³²P dATP 103.3 H₂O

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00023

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Red Tag "opt" field

1. White @ 5 u/ μ l2. Red 15 (100 μ l)~~10 μ l 5/4 Dye~~~~70 μ l H₂O~~

2x

5/4 Dye 8 μ l Dye10X + Mg 14.67 μ l10X - Mg 5.33 μ lH₂O 72 μ l1.1 ml
MgCl₂3. Red 25 (100 μ l)

5/4 Dye 13.33

50% Ery 2.67

10X + Mg 10.67

10X - Mg 9.33

0.8 ml
MgCl₂

Witnessed & Understood by me,

Gu

Date

Invented by

Recorded by

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10x 2x
~~5x~~ 5x dge
2x 10x

0.16 0.4 0
4 2.67 0
16 17.33 20

(15^{at}) 10x
1 2 3 4 5 6 7 8 9 10 11 12
15 13 11 9 8 7 6 5 4 3 2 0
+ Mg 20 17.33 14.67 12 10.67 9.33 8 6.67 5.33 4 2.67 0
- Mg 0 2.67 5.33 8 9.33 10.67 12 13.33 14.67 16 17.33 20

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

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[Mg] app -

	1	2	3	4	5	6	7	8	9	10	11	12
[Mg]	1.5	1.3	1.1	0.9	0.7	0.5	0.3	0.2	0			
	1.5	1.3	1.1	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	
					0.7	0.6	0.5	0.4	0.3	0.2	0.1	0

10X

10X Buffers

~~200~~ 500 μ l 1003 2.6 2.2 1.8 1.4 ~~1.2~~ 1.2 1 0.1

10X T Mg

10 8.67 7.33 6 4.67 8 6.67 5.33

10X - Mg

0 4.33 2.67 4 5.33 2.67 5.33 8 10.67 12 13.33 14

~~50~~ red - (120 μ l)9.6 μ l 5/4 dye 10.4 H₂O(H₂O) white

12

108

white

12 50% gly, 108 H₂O2X Emix (275 μ l)5.5 Tag (50 μ l), 11 μ l ea pm λ - 27.5 μ l, dATP's 5.5 27.5, 209.55 μ l dATP

To Page No. _____

Witnessed & Understood by me,

L. J. J.

Date

Invented by

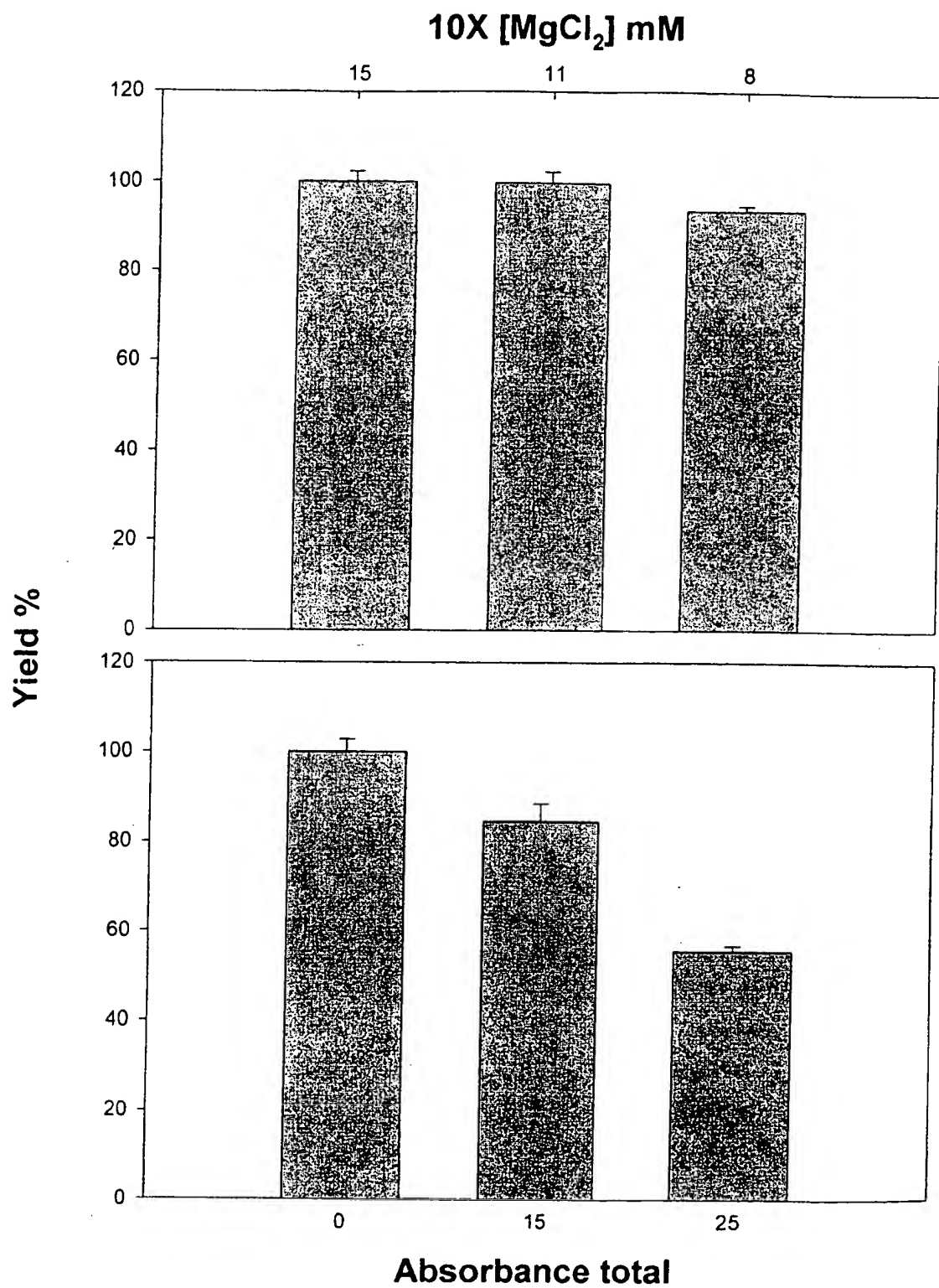
L. J. J.

Date

Recorded by

David Red

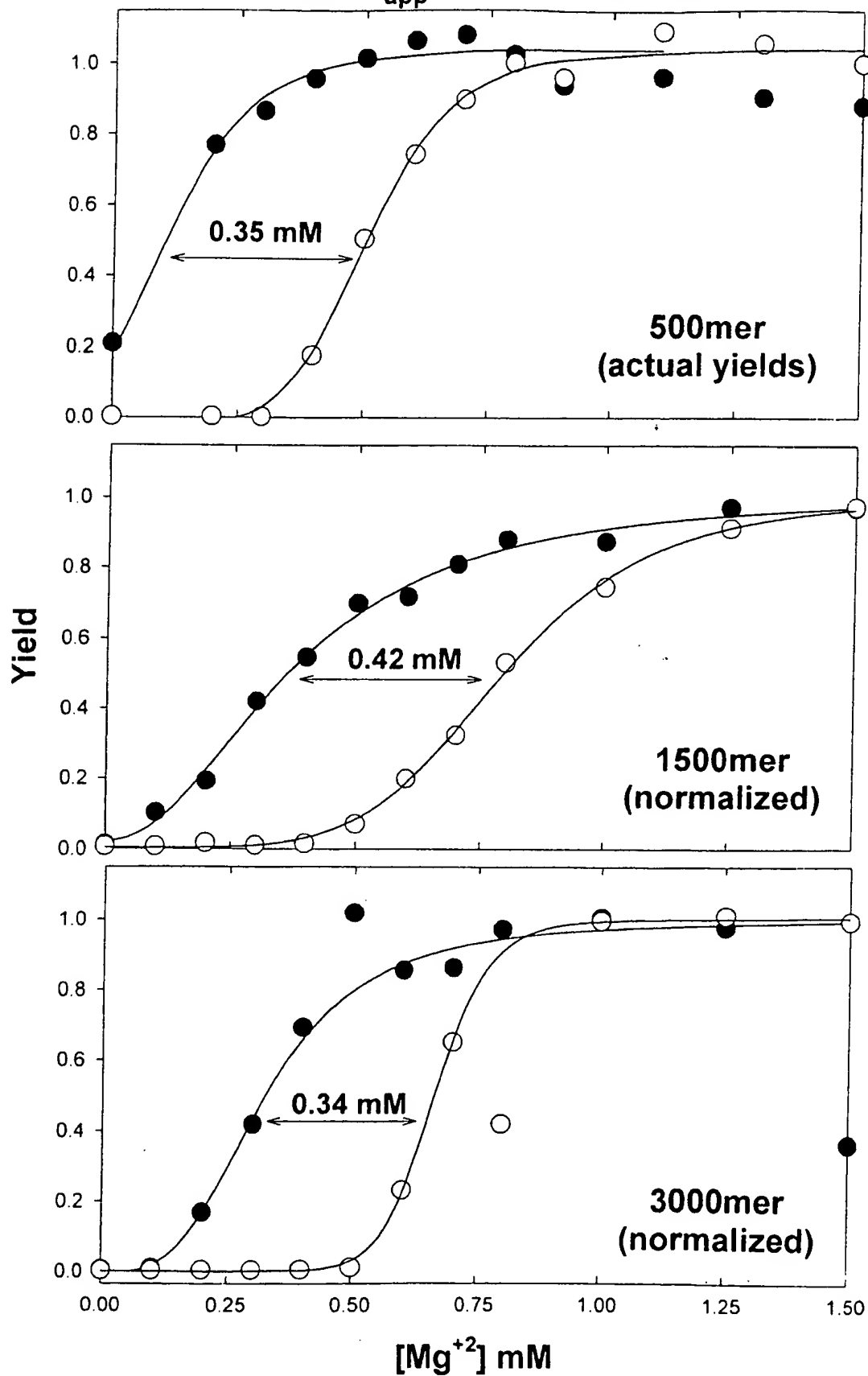
Yield of 500mer and 3000mer for RedTaq compensated for $[Mg^{+2}]$



USER: 1 ID: BONEHEAD PRESET TIME: 1.00
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N RS232: N
 H#: 0 AOC: N BCF: N RCM: N
 CHANNEL 1-LL: 0 UL: 1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
 DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000
 HALF LIFE (DAYS): N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	ERR
1	**	1	567820.00	1.19	0.05	0.22	
2	**	2	592040.00	1.16	0.05	0.61	
3	**	3	535880.00	1.22	0.05	0.98	
4	**	4	579340.00	1.17	0.05	1.36	
5	**	5	580220.00	1.17	0.05	1.73	
6	**	6	548380.00	1.21	0.05	2.11	
7	**	7	594480.00	1.16	0.05	2.48	
8	**	8	563900.00	1.19	0.05	2.86	
9	**	9	546180.00	1.21	0.05	3.23	
10	**	10	266920.00	1.73	0.05	3.61	
11	**	11	268040.00	1.73	0.05	3.97	
12	**	12	245600.00	1.80	0.05	4.33	
13	**	1	585680.00	1.17	0.05	4.78	
14	**	2	480660.00	1.29	0.05	5.16	
15	**	3	321860.00	1.58	0.05	5.53	
16	**	4	613700.00	1.14	0.05	5.90	
17	**	5	526320.00	1.23	0.05	6.27	
18	**	6	336100.00	1.54	0.05	6.65	
19	**	7	585660.00	1.17	0.05	7.02	
20	**	8	497640.00	1.27	0.05	7.40	
21	**	9	329180.00	1.56	0.05	7.77	
22	**	10	421780.00	1.38	0.05	8.14	
23	**	11	358580.00	1.49	0.05	8.52	
24	**	12	221420.00	1.90	0.05	8.88	

Apparent $[Mg^{2+}]$ of red taq.
 $[Mg^{+2}]_{app} = 0.37 \pm 0.04$ mM



1500 bp λ

SER: 1 ID: BONEHEAD PRESET TIME: 1.00
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N RS232: N
 H#: 0 AQC: N QCF: N RCM: N
 CHANNEL 1-LL: 0 UL: 1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
 DATA CALC: CPM. UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000
 HALF LIFE(DAYS): N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	10xEmg3	ERR
1	**	1	37715.00	1.86	0.20	0.38	15	—
2	**	2	57755.00	1.86	0.20	0.90	12.5	
3	**	3	51535.00	1.96	0.20	1.42	10	
4	**	4	52230.00	1.96	0.20	1.95	8	
5	**	5	48012.00	1.83	0.25	2.52	7	Red
6	**	6	42568.00	1.94	0.25	3.10	6	
7	**	7	41480.00	1.96	0.25	3.67	5	
8	**	8	32380.00	1.88	0.35	4.35	4	
9	**	9	24697.78	1.89	0.45	5.13	3	
10	**	10	11444.44	1.97	0.90	6.37	2	
11	**	11	6144.00	2.55	1.00	7.71	1	
12	**	12	735.00	7.38	1.00	9.04	0	—
13	**	1	71333.33	1.93	0.15	9.59	15	
14	**	2	66820.00	2.00	0.15	10.07	12.5	
15	**	3	54565.00	1.91	0.20	10.58	10	
16	**	4	38780.00	1.85	0.30	11.21	8	
17	**	5	23611.11	1.94	0.45	11.99	7	
18	**	6	14545.71	1.98	0.70	13.02	6	White
19	**	7	5161.00	2.78	1.00	14.35	5	
20	**	8	1119.00	5.98	1.00	15.68	4	
21	**	9	756.00	7.27	1.00	17.02	3	
22	**	10	1225.00	5.71	1.00	18.36	2	
23	**	11	560.00	8.45	1.00	19.70	1	
24	**	12	546.00	8.56	1.00	21.04	0	—

5000 by 2

ID: BONEHEAD PRESET TIME: 1.00
 REPEAT: 1 CYCLE REPEAT: 1 SCR: N RS232: N
 O ADC: N QCF: N RCN: N
 CHANNEL 1-LL: 0 UL: 1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
 DATA CALC: CPM. UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000
 HALF LIFE (DAYS): N

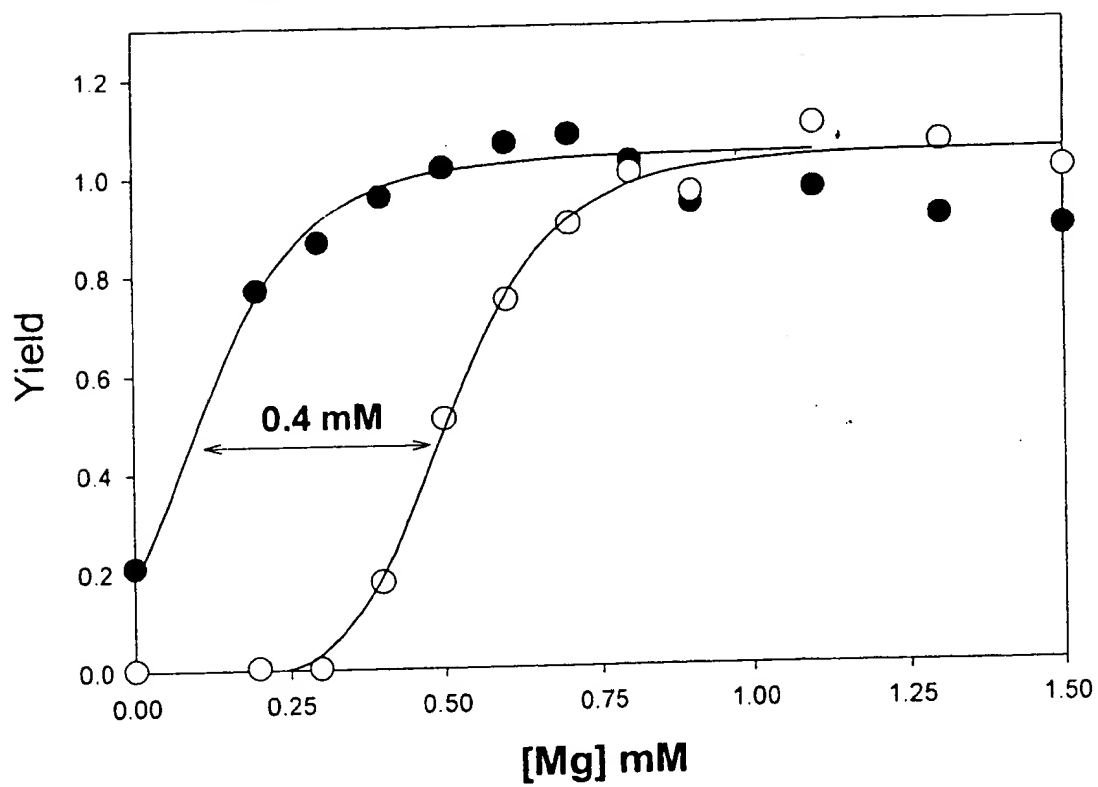
SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	ERR
1	**	1	27832.50	1.90	0.40	0.58	15
2	**	2	75666.66	1.88	0.15	1.06	12.5
3	**	3	77986.66	1.85	0.15	1.53	10
4	**	4	75440.00	1.88	0.15	2.00	8
5	**	5	67020.00	1.99	0.15	2.47	7
6	**	6	66455.00	1.73	0.20	2.99	6
7	**	7	79033.33	1.84	0.15	3.47	5
8	**	8	53850.00	1.93	0.20	3.99	4
9	**	9	32408.57	1.88	0.35	4.67	3
10	**	10	13086.25	1.95	0.80	5.80	2
11	**	11	861.00	6.82	1.00	7.13	1
12	**	12	497.00	8.97	1.00	8.47	0
13	**	1	86740.00	1.75	0.15	9.00	15
14	**	2	88366.66	1.74	0.15	9.48	12.5
15	**	3	87173.33	1.73	0.15	9.95	10
16	**	4	36680.00	1.91	0.30	10.58	8
17	**	5	57195.00	1.87	0.20	11.10	7
18	**	6	20180.00	1.99	0.50	11.93	6
19	**	7	999.00	6.33	1.00	13.27	5
20	**	8	440.00	30.15	0.10	13.68	4
21	**	9	420.00	30.86	0.10	14.13	3
22	**	10	470.00	29.17	0.10	14.55	2
23	**	11	335.00	24.43	0.20	15.08	1
24	**	12	300.00	29.81	0.15	15.55	0

10X [mg]

red

white

Apparent $[Mg^{2+}]$ of red taq.
Red curve is white curve displaced by 0.4 mM



D: BONEHEAD PRESET TIME: 1.00
 REPEAT: 1 CYCLE REPEAT: 1 SCR: N RS232: N
 ADC: N QCF: N RCM: N
 CHANNEL 1-LL: 0 UL: 1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2516: 0.00 LSR: 0
 DATA CALC: CPM. UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000
 ALF LIFE(DAYS): N

AM	FDS	CH	CPM	2SIG%	TIME	EL TIME	[Mg] mM	ERR
1	**	1	362180.00	1.49	0.05	0.22	1.5	
2	**	2	372200.00	1.47	0.05	0.60	1.3	
3	**	3	396460.00	1.42	0.05	0.97	1.1	
4	**	4	385060.00	1.44	0.05	1.35	0.9	
5	**	5	420960.00	1.38	0.05	1.72	0.8	
6	**	6	443840.00	1.34	0.05	2.10	0.7	
7	**	7	437640.00	1.35	0.05	2.47	0.6	
8	**	8	417680.00	1.38	0.05	2.85	0.5	
9	**	9	393960.00	1.43	0.05	3.22	0.4	
10	**	10	355980.00	1.50	0.05	3.60	0.3	
11	**	11	316460.00	1.59	0.05	3.97	0.2	
12	**	12	86620.00	1.75	0.15	4.45	0	
13	**	1	411420.00	1.39	0.05	4.93	1.5	
14	**	2	434200.00	1.36	0.05	5.30	1.3	
15	**	3	449840.00	1.33	0.05	5.68	1.1	
16	**	4	394500.00	1.42	0.05	6.05	0.9	
17	**	5	411460.00	1.39	0.05	6.43	0.8	
18	**	6	369000.00	1.47	0.05	6.80	0.7	
19	**	7	306420.00	1.62	0.05	7.18	0.6	
20	**	8	208220.00	1.96	0.05	7.53	0.5	
21	**	9	72633.33	1.91	0.15	8.01	0.4	
22	**	10	1056.00	12.31	0.25	8.58	0.3	
23	**	11	1575.00	11.27	0.20	9.11	0.2	
24	**	12	1208.33	7.43	0.60	10.06	0	

Page No. _____

PCR yields

Compare red @ 1 μ /ul with
white @ 5 μ /ul

1. 3X red/buffer

agg = 45, buffer = 3X

10X Buffer = 11.1 mM $MgCl_2$

10X (200 μ l) = 153.33 10X + Mg
40.67 10X - Mg

3X- (200 μ l) 24 μ l 5/4,

48.88 μ l 10X + Mg

11.11 μ l 10X - Mg

116 H_2O

3X White Buffer 60 μ l 10X + Mg, 190 H_2O

3X PMR 2-6 50 μ l ea 3 μ l pmr
47 μ l H_2O

3X Enz - 275 μ l λ PMR.1 - 16.5 μ l

λ ~~275~~ μ l 13.5 μ l

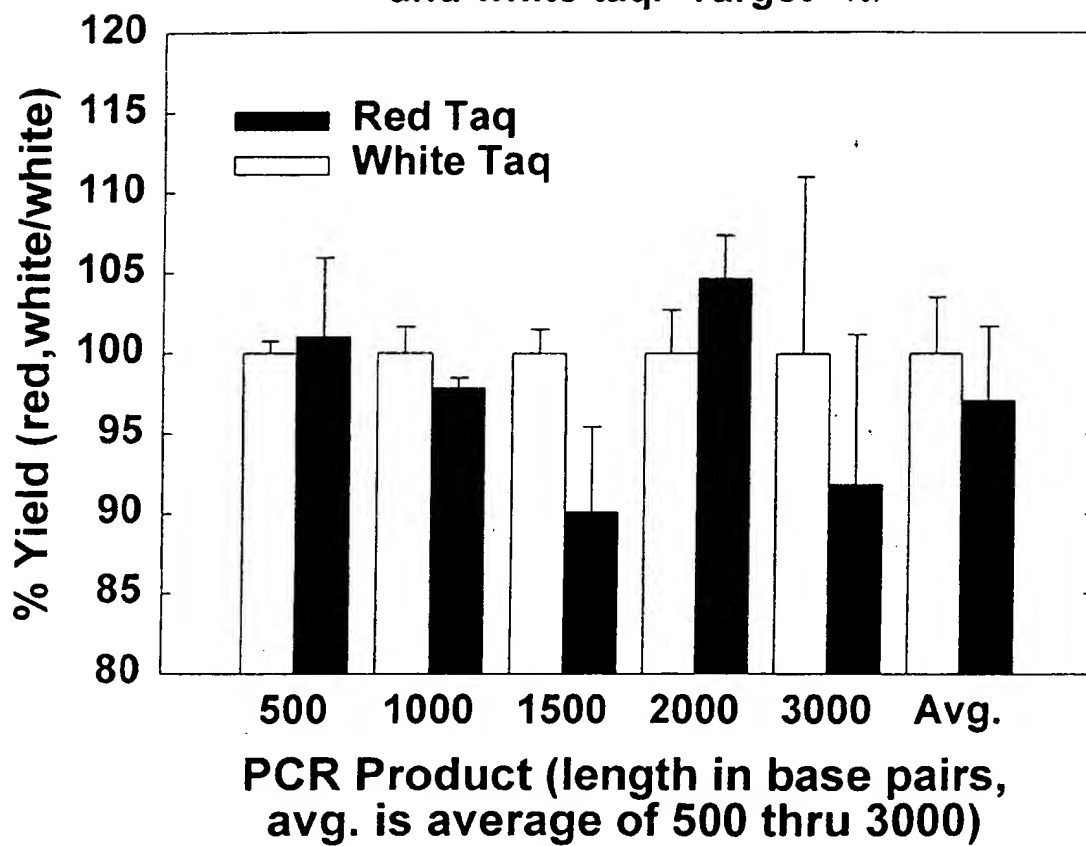
10 mM dNTP's 8.25 μ l, Tag 8.25

$\alpha^{32}P$ dCTP 5 μ l, 228.5 H_2O

To Page No. _____

Erin Reed

PCR product yields using red
and white taq. Target = λ .



yield

ER: 1 ID: BONEHEAD PRESET TIME: 1.00
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N RS232: N
H#: 0 AGC: N DCF: N REM: N
CHANNEL 1-LL: 0 UL: 1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0
DATA CALC: CPM. UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000
HALF LIFE (DAYS): N

SAM	PUS	CH	CPM	2SIG%	TIME	EL TIME	ERR
1 **	1	1	924380.00	0.93	0.05	0.23 R	-
2 **	2	1	956780.00	0.91	0.05	0.62 W	- 500
3 **	3	1	1019260.00	0.89	0.05	1.00 R	-
4 **	4	1	1031520.00	0.88	0.05	1.38 W	- 1000
5 **	5	1	780480.00	1.01	0.05	1.77 R	-
6 **	6	1	820460.00	0.99	0.05	2.15 W	- 1500
7 **	7	1	381360.00	1.45	0.05	2.53 R	-
8 **	8	1	357640.00	1.50	0.05	2.90 W	- 2000
9 **	9	1	1012000.00	0.89	0.05	3.28 R	-
10 **	10	1	1141220.00	0.84	0.05	3.67 W	- 3000
13 **	1	1	969840.00	0.91	0.05	4.15	.
14 **	2	1	958300.00	0.91	0.05	4.53	.
15 **	3	1	1023520.00	0.88	0.05	4.92	.
16 **	4	1	1066120.00	0.87	0.05	5.30	.
17 **	5	1	767360.00	1.02	0.05	5.68	.
18 **	6	1	844480.00	0.97	0.05	6.07	.
19 **	7	1	377740.00	1.46	0.05	6.44	.
20 **	8	1	377360.00	1.46	0.05	6.82	.
21 **	9	1	1047740.00	0.87	0.05	7.20	.
22 **	10	1	925920.00	0.93	0.05	7.58	.

10x Red = 11 mM Mg

SER: 1 ID: BONEHEAD PRESET TIME: 1.00
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N RS232: N
 HH: 0 ABC: N DCF: N RCM: N
 CHANNEL 1-LL: 0 UL: 1000 2SIGMA: 2.00 PKG SUB: 0.00 PKG 2SIG: 0.00 LSR: 0
 DATA CALC: CPM. UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000
 HALF LIFE (DAYS): N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	ERR
1	**	1	1019620.00	0.89	0.05	0.23	
2	**	2	969680.00	0.91	0.05	0.62	
3	**	3	1032600.00	0.88	0.05	1.01	
4	**	4	1045260.00	0.87	0.05	1.39	
5	**	5	697540.00	1.07	0.05	1.77	
6	**	6	826540.00	0.98	0.05	2.15	
7	**	7	396480.00	1.42	0.05	2.52	
8	**	8	368860.00	1.47	0.05	2.90	
9	**	9	860500.00	0.96	0.05	3.28	
10	**	10	1112760.00	0.85	0.05	3.67	

Originated

Red Taq preparation.

1. **Prepare Mg Acid Red 1 and Mg Acid Violet solutions.** Dissolve dyes in *Taq* dilution buffer at approximately 10 mg/ml, 0.2 μ m filter. Note: dissolution of Acid Red 1 generally requires heating in a hot (90°C or above) water bath.
2. **Measure solution absorbances.** To a tared 15 ml conical tube add approximately 10 ml of water, record weight of water (w). Add approximately 10 μ l of dye solution and record weight (d). Cap and mix. Calculate dilution factors (f). $f = 1.1423 \times d / (d - w)$. 1.1423 is *Taq* dilution buffer density. Measure absorbance spectrum for each diluted dye solution. Record absorbance (A_{AR1} and A_{AV2} for acid red 1 and acid violet 5 respectively) of each solution at their respective λ_{max} . Acid Red 1 \approx 532 nm, Acid Violet 5 \approx 527 nm.
3. **Calculate absorbance of dye concentrates.** $A_c = A_d \times f$, A_c is concentrate absorbance in absorbance units per ml.
4. **Calculate Red *Taq* dilution volumes.** The final solution will have *Taq* at 1 u/ μ l, Acid Red 1 at 240 abs. units per ml and Acid Violet 5 at 60 abs. units per ml. For v ml of solution the final weight (wf) will be $1.1423 \times v$. The solution will contain: $(240/A_{AR1}) \times wf$ of Acid Red 1, $(60/A_{AV2}) \times wf$ of Acid Violet 5, $0.8 \times wf - ((240/A_{AR1}) \times wf + (60/A_{AV2}) \times wf)$ of *Taq* dilution buffer and $0.2 \times wf$ of *Taq*.
5. **Prepare solution.** The solution is prepared in a sterile environment (i.e. hood) by weighing the components into a tared sterile container. The container will depend on the size of the lot being produced. If possible, solutions should be added to a capable bottle, i.e. conical tubes, Nalgene bottles etc. Thus, on a balance in a laminar flow hood, carefully weigh each component into the tared container. Record the weight of each addition. Retare after each addition. Solution additions can generally be within $\pm 1\%$. Thoroughly mix after the last component is added.
6. **Perform Suitability assay.** PCR products (λ and 500mer primers, QC SOP) using Red *Taq* and 10X Red *Taq* buffer should be indistinguishable from *Taq* (D1806) and 10X PCR buffer.